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ORIGINAL PAPERS

Biosorption of U, La, Pr, Nd, Eu and Dy by *Pseudomonas aeruginosa*

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Kinetic studies with *Pseudomonas aeruginosa* using actinides and lanthanides indicated a two-phase metal uptake. Equilibrium uptake data of all the metals studied could be fitted to Langmuir as well as Freundlich models. The Scatchard plots showed that there were mainly two types of receptor sites on the cell walls of *P. aeruginosa* having different affinities for the metal ions. EDAX studies revealed replacement of calcium and magnesium ions from biomass by sorbed metal. Around 85% of the adsorbed metal could be released using citrate buffer (pH 4.0, 0.2 M). Metal desorption was as high as 95% with HCl. Continuous flow studies using *P. aeruginosa* immobilized on activated alumina gave 80% enhancement of lanthanum removal efficiency compared to the control column. Regeneration of the column resulted in 80% of its initial capacity in succeeding cycles. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 1–7.

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Introduction

Heavy metals and radionuclides remain as alarming pollutants due to their nondestructive nature, toxicity, bioaccumulation and subsequent biomagnification. Environmental biotechnology has identified the potential of some microbes to accumulate metals to levels far exceeding their metabolic requirements. A practical application of this property of microorganisms is their use in the treatment of radioactive waste and polluted repository sites [3]. Bacteria are of particular interest because their surface/volume ratio is relatively high, especially in the case of nonspherical forms [4,6,15].

Biosorption, in which microbes are directed to accumulate metals from aqueous solutions, has now evolved into a commercial process. Commercial organizations like B.V Sorbex Inc., Canada, Bio-recovery Systems, USA [16] and Advanced Mineral Technologies Inc., USA have developed biosorbents for a spectrum of heavy metals [5]. However, only a few reports are available on the use of biosorbents for removal and recovery of lanthanides.

Andres *et al.* [1] studied the biosorption potential of *Mycobacterium smegmatis*. They compared the uptake of various actinides and lanthanides by dried cells of *M. smegmatis*. Muraleedharan *et al.* [12] reported the treatment of a simulated monazite processing industry effluent by using a nonviable wood-rotting macro fungus, *Ganoderma lucidum*. Most of these commercially available biosorbents employ nonviable microbes. *Pseudomonas aeruginosa* is reported to have very high metal uptake capacity, especially for uranium [15]. However no work has been reported on the use of live *P. aeruginosa* for the removal of rare-earth elements.

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The main objective of the present investigation was to evaluate the potential of viable *P. aeruginosa* for removal of rare earths and to investigate the suitability of a continuous flow immobilized microbial reactor for lanthanum uptake.

Materials and methods

Microbes

A pure culture of *P. aeruginosa* MTCC-1223 was used for this study. The strain was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India.

Metals

Uranium (U) was used as a model metal for actinides and lanthanum (La), praseodymium (Pr), neodymium (Nd), europium (Eu), and dysprosium (Dy) were selected from lanthanides. Chloride salts of lanthanides were used. Column studies using immobilized *P. aeruginosa* were conducted only with lanthanum.

Matrices for microbial immobilization

The matrices selected for microbial immobilization were commonly available and inexpensive. They included activated alumina, coconut shell, Giridih bituminous coal, glass, ceramic material, refractory bricks, rice husks, silica gel (1-mm diam), and sand. All except silica gel were pulverized and sieved to a size range of 0.4–0.6 mm.

Metal uptake studies

P. aeruginosa was grown in nutrient medium M_1 (peptone, 10 g/l; NaCl, 5 g/l; yeast extract, 2 g/l; beef extract 1 g/l) for 24 h before collecting cells by centrifugation at $6000 \times g$ for 10 min. Cells were

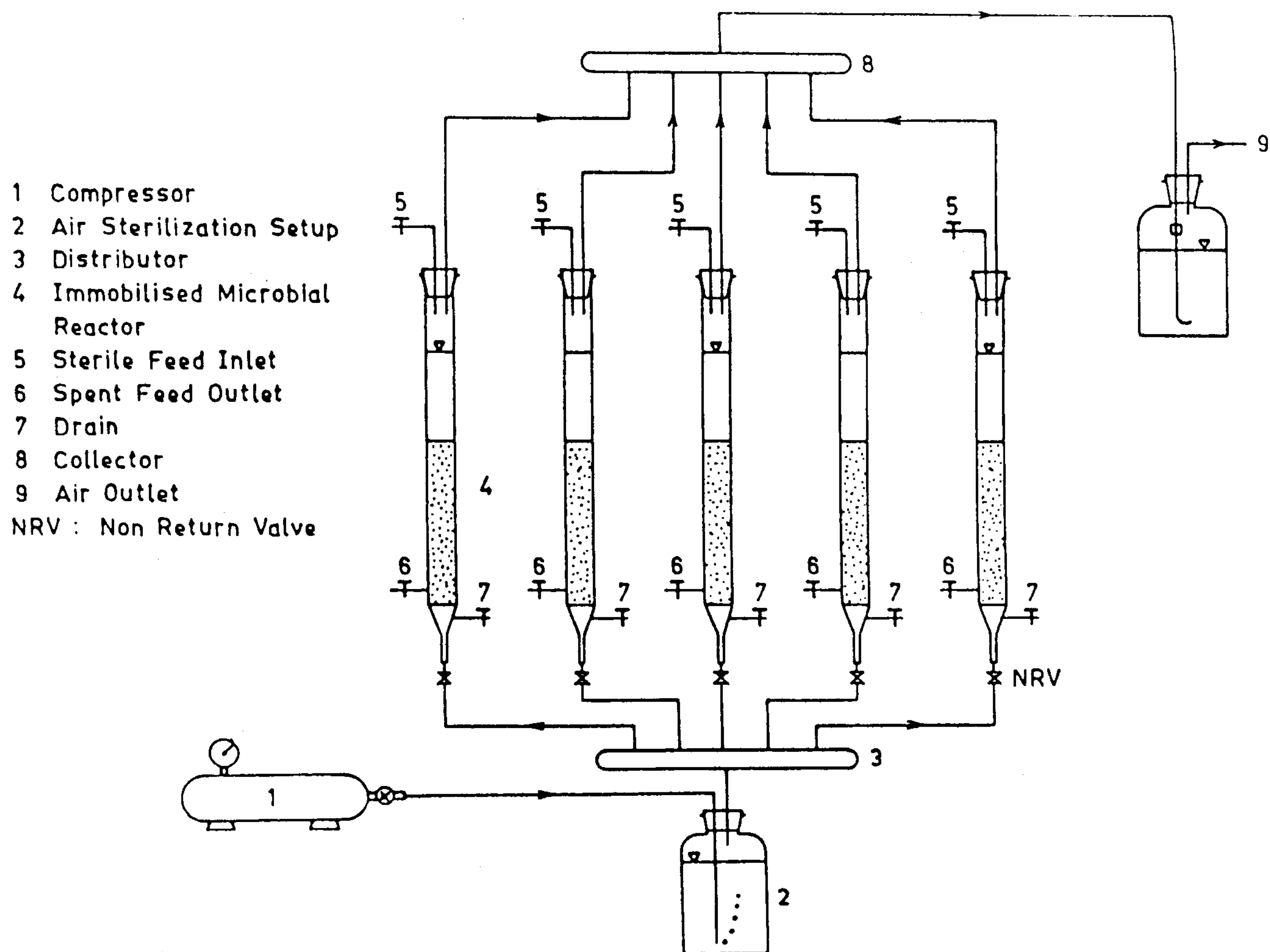

Figure 1 Set-up for bacterial immobilization.

separated by centrifugation at 6000×*g* for 10 min and washed three times in physiological saline. One hundred milliliters of uranium solution (120 mg/l) was taken in 250-ml conical flasks. Harvested bacterial cells were transferred to the metal solution and pH was maintained at 5.0 by adding 1 ml of 1 M acetate buffer. The flasks were kept in the environmental shaker (New Brunswick Scientific, USA) maintained at 30°C. Samples were withdrawn and analyzed for residual uranium concentrations in the supernatant after separating the bacterial cells by centrifugation at 6000×*g* for 10 min.

Table 1 Kinetics of uranium uptake by *P. aeruginosa*

Time, h	Residual U conc., mg/l
Data set 1	
0.00	120.0
0.25	80.0
0.50	70.0
1.00	65.0
Data set 2	
2.00	62.0
3.00	61.0
4.00	60.0

Equilibrium studies

Adsorption equilibrium experiments were conducted using U, La, Nd, Pr, Eu and Dy, in the concentration range of 0.25 to 2 mM. The reaction mixture consisted of 100 ml adsorbate solution buffered at pH 5.0 with acetate buffer and a known bacterial concentration. The reaction time was maintained at 4 h. The mixture was agitated at 30 rpm in an end-on-end rotary shaker (Widson, New Delhi, India). Subsequently sorbate and sorbent were separated by centrifugation. The supernatants were analyzed for metal concentration.

Batch desorption studies

Batch desorption studies were conducted after the adsorption of La^{3+} *by P. aeruginosa*. The metal-loaded cells were collected by centrifugation, and were suspended in 25 ml of the desorbing solution containing each of the eluants: 0.2 M acetate, 0.2 M citrate, 0.2 M HCO_3^- and 0.2 M HCl.

Immobilization of bacterial cells on solid matrices

The experimental set-up for immobilization of bacterial cells on solid matrices is presented in Figure 1. The entire set-up was disinfected by passing potassium permanganate solution through it followed by washing it with sterile distilled water. Before immobilization, the matrices were soaked in water for 48 h, dried

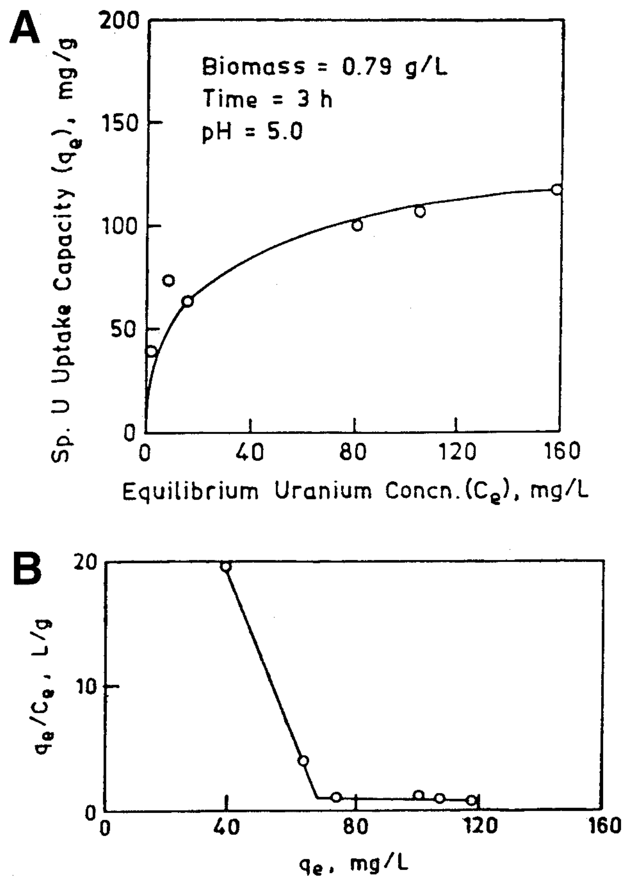

Figure 2 (A) Equilibrium curve for sorption of U on *P. aeruginosa*. (B) Scatchard plot for sorption of U on *P. aeruginosa*.

and sterilized by autoclaving. About 100 g of each matrix (five at one time) were transferred into separate columns along with 150 ml (1 g dry weight of bacteria/l) of a 24-h-old culture of *P. aeruginosa*. Sterilized air was passed continuously through the reactor. The spent nutrient medium (M1) was replaced once in 2 days with fresh sterile nutrient medium. Every fifth day, 1 g of immobilized medium was removed from each column for determination of cell density. The cell mass immobilized on each matrix was determined by total protein determination [9] and converted to cell density using a calibration curve. An appropriate control was included for protein estimation. These controls were the corresponding immobilization matrix without any microbes, which eliminated the contribution or interference by the matrices. This procedure was continued till a steady state was attained in terms of immobilized cell density. After this, the matrices were washed three times in physiological saline and stored at 0–4°C.

Column studies

Bench scale column studies were conducted with viable immobilized microbes using lanthanum as adsorbate. Glass columns having 25 mm diameter and 500 mm length were used as reactors. Bed depth and flow rate was maintained at 300 mm and 2.25 $m^3/m^2/h$ respectively. Influent concentration was kept at 0.75 mM of La(III), and pH 5.0 using acetate buffer. Columns were operated in upflow mode. An appropriate control column having immobilization matrix (activated alumina) as adsorbent was operated under identical conditions.

After the column was exhausted, it was regenerated using 0.2 M citrate buffer (pH 4) equal to 1/50 the throughput volume at a flow rate of 2.25 $m^3/m^2/h$ (18 ml/min) followed by a distilled water wash. To estimate the immobilized microbial cell's viability and density, dehydrogenase activity and total protein content were measured before and after column studies.

Analytical procedures

Uranium was quantified using the spectrophotometric method of Mercenko [10] with Arsenazo-III as the colour-developing agent. Rare-earth elements were analyzed using a colorimetric method developed by Omishi and Sekine [14]. Cell protein was determined according to the method of Herbert *et al.* [7]. This method is a modification of the method of Lowry *et al.* [9]. Assay of INT-dehydrogenase activity was done using the method developed by Awong *et al.* [2].

EDAX analyses were conducted using a Kevex apparatus attached to the SEM (JEOL, Japan). The accelerating voltage was constant at 15 kV to facilitate emission of secondary X-rays. Samples were dehydrated by multiple washes in increasingly concentrated acetone. Samples were then fixed onto a graphite or aluminium stub (8 mm) using absolute acetone, graphite paint or silver paste. Stubs were then kept in a PS-2 silver sputtering unit

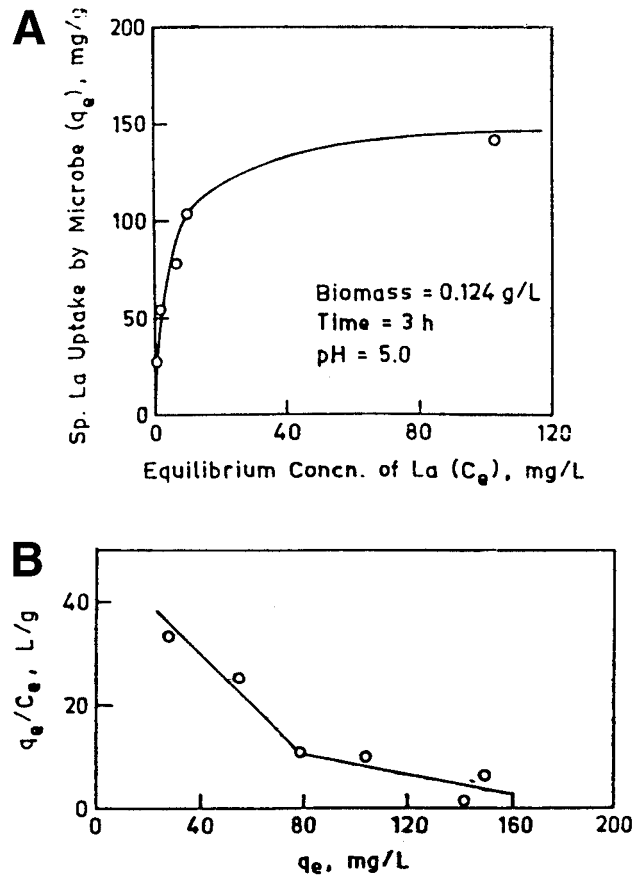

Figure 3 (A) Equilibrium curve for sorption of La on *P. aeruginosa*. (B) Scatchard plot for sorption of La on *P. aeruginosa*.

(International Scientific Instruments, USA) and a 40-Å coating of silver was applied under vacuum for 15 min.

Results and discussion

Sorption kinetics

Biosorption is reportedly a fast process, with most of the mass transfer taking place within a few hours [11]. The kinetic data of uranium uptake by *P. aeruginosa* showed two distinct adsorption phases: an initial rapid uptake (data set 1 in Table 1) followed by gradual uptake (data set 2 in Table 1), with equilibrium attained at 4 h.

Extracellular adsorption is generally by ion-exchange processes, which are fast reactions. The initial rapid uptake is probably entirely due to the biosorption of metal ions onto the cell wall. The second phase may be due to the biologically mediated transfer of metal ions into the cytoplasm. Such cell-mediated transfer is a slow process and is of importance if the biomass is to be applied in a continuous reactor. Similar biphasic metal uptake was observed by Andrews *et al.* [1] for biosorption of actinides and lanthanides by nonviable *M. smegmatis*.

Adsorption equilibria studies

Both lanthanides and actinides belong to the so-called oxygen-seeking group of metals, as per the definition of Nieboer and Richardson [13]. Thus, the behavior of these metals toward the microbial cells may be similar. Adsorption equilibrium studies of lanthanides were conducted using *P. aeruginosa* and the data were tested for three models defined by the Freundlich, Langmuir and Brunauer–Emmett–Teller (BET) isotherms, which are given in Equations 1–3 respectively.

$$\frac{1}{q_e} = \frac{1}{Q_{\max}} + \frac{1}{Q_{\max} b C_e} \tag{1}$$

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \tag{2}$$

$$q_e = \frac{B C_e Q^o}{(C_s - C_e)\left[1 + (B-1)\left(\frac{C_e}{C_s}\right)\right]} \tag{3}$$

where q_e is the sorption capacity (mg/g); C_e is the metal concentration in solution (mg/l), at equilibrium; Q, b, K, n, and B are constants. C is the saturation concentration of solute, Q is the number of moles of solute adsorbed per unit weight of adsorbent in forming a complete monolayer on the surface.

The equilibrium sorption curves for U and La are presented in Figures 2(a) and 3(a). In both cases, adsorption followed both Langmuir and Freundlich isotherms. Interestingly, none of the experimental data followed the linearised BET model which is a characteristic of multilayer adsorption. The $Q_{\max}$ values obtained from Langmuir plots for different metals are presented in Table 2.

Table 2 Maximum specific uptake capacity ($Q_{\max}$, mM/g) of *P. aeruginosa* for uranium and lanthanides

Metal	Q, mM/g
U	0.62
La	1.00
Pr	0.94
Nd	1.10
Eu	0.83
Dy	1.00

Table 3 Scatchard plot slopes of uranium and lanthanides uptake by *P. aeruginosa*

Metal	K_1	K_2
U	0.558	0.006
La	0.246	0.129
Pr	0.287	0.124
Nd	0.211	0.045
Eu	1.389	0.052
Dy	1.964	0.040

The bacterial cell wall has a heterogeneous surface. Amino, phosphate, sulfhydryl, carboxyl and hydroxyl groups are major potential adsorption sites for metal ions [13]. Each functional group exhibits a different specificity toward metal ions.

A Scatchard plot featuring the variation of q_e as a function of q_e/C_e describes the interaction between receptor sites and binding ions. In both cases (Figures 2(b) and 3(b)), Scatchard plots gave two intersecting lines which are the tangents of the curves. The slopes K_1 and K_2 values derived from Scatchard plots are presented in Table 3. It is clear that there are mainly two types of receptor sites on the cell wall of *P. aeruginosa* which exhibit different affinities toward metal ions. Similar observations were made by Huang *et al.* [8] for the sorption of Cu by *S. cerevisiae* and Andres *et al.* [1] for uptake of actinide and lanthanide ions by *M. smegmatis*.

Ligand exchange and desorption studies

Desorption of metal from metal-loaded biosorbents may provide some insight into the extent of metal ion penetration into the cell structure. In the case of nonviable cells, elution of total accumulated metal can be achieved by acid digestion [8]. However, in the case

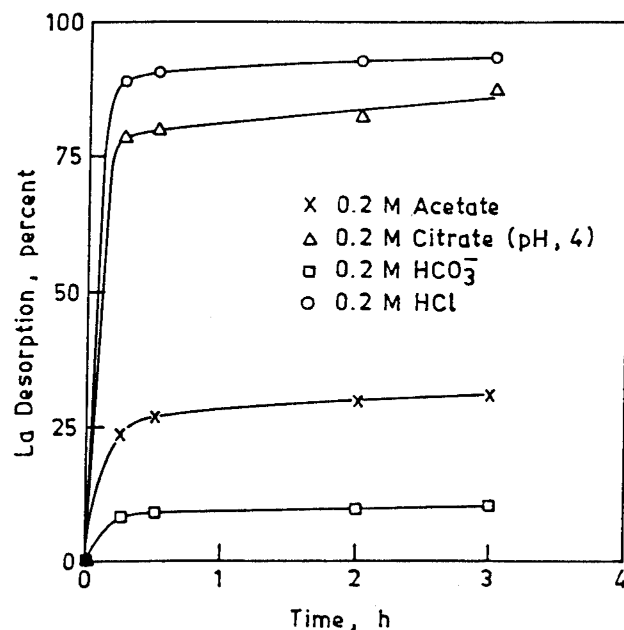

Figure 4 Desorption kinetics of lanthanum from *P. aeruginosa*.

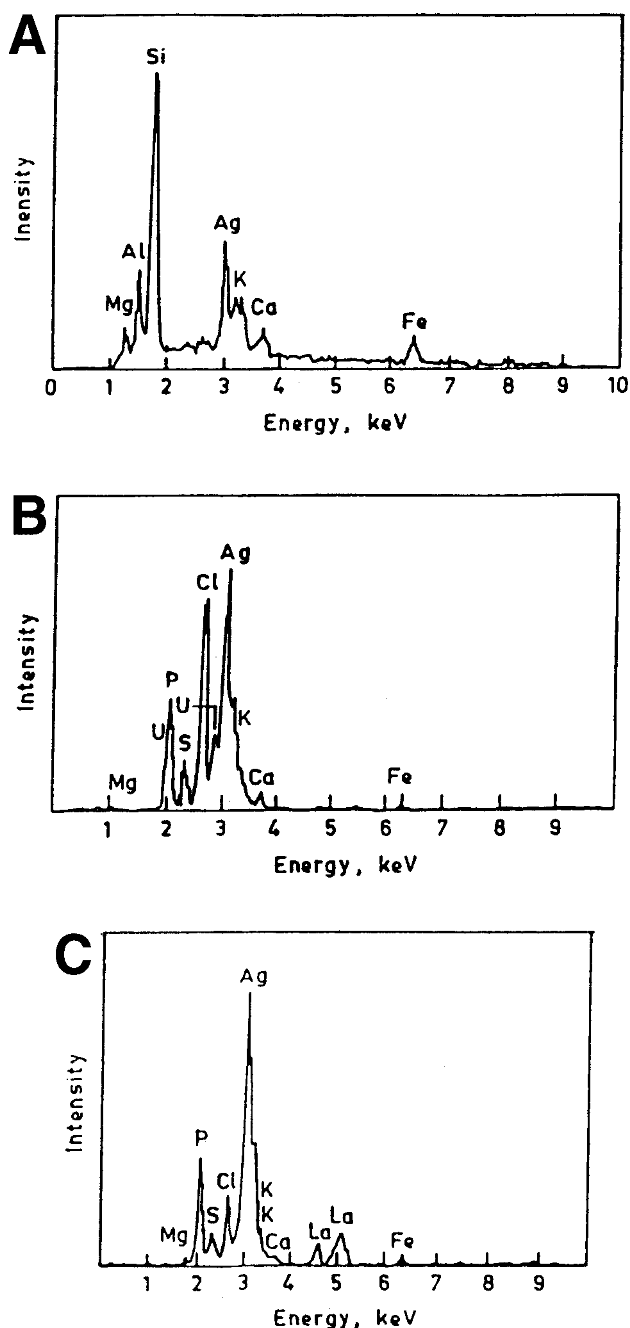

Figure 5 (A) EDAX spectrum of *P. aeruginosa* before adsorbing U. (B) EDAX spectrum of *P. aeruginosa* after adsorbing U. (C) EDAX spectrum of *P. aeruginosa* after adsorbing La.

of living cells, the desorption conditions to which the microbes can be subjected are restricted if viability is maintained [17]. Desorption studies were conducted using La as a model metal for lanthanides.

As acetate and bicarbonate are reported to be eluents for uranium desorption [11], they were used in addition to citrate and HCl to desorb La sorbed on *P. aeruginosa*. The results are presented in Figure 4. Acid desorbed almost 95% of the metal bound to the cells. However, the viability of *P. aeruginosa* exposed to acid was affected. Citrate buffer (0.2 M, pH 4.0) desorbed about 80% of the sorbed metal without affecting viability. Acetate and bicarbonate desorbed La to a lesser extent. Decreased desorption by these ligands can be due to stronger interaction between cell surface groups and the metal, compared to the metal and desorbents.

To check viability, INT dehydrogenase activity of the cells was determined after desorption. Cells subjected to acetate and bicarbonate exhibited above 90% of their initial activity whereas cells exposed to citrate showed only 85%. *P. aeruginosa*, which came in contact with HCl did not retain INT-dehydrogenase activity.

EDAX analysis

X-ray energy dispersion analysis (EDAX) is useful for the study of the chemical and elemental characteristics of the adsorbent. *P. aeruginosa* was subjected to EDAX analysis before and after lanthanum sorption. The results are presented in Figure 5. The EDAX spectrum of the biosorbent before metal uptake exhibits distinct calcium and magnesium peaks indicating the presence of substantial amounts of these metals in the adsorbent. After lanthanum uptake, these two peaks are much smaller, and the lanthanum signal is distinctive. This indicates the possibility of lanthanum displacing calcium and magnesium from the cellular material. The results are only indicative and no attempt has been made to quantify the findings in the preliminary work on EDAX.

Screening of matrices based on immobilized cell density

Separation of metal-loaded biomass requires an energy-intensive solid–liquid separation process such as centrifugation which is the major limitation for practical application of the process. In order to eliminate this problem, an attempt was made to immobilize the microbes on suitable matrices before studying the efficiency of the process.

Effective and economic immobilization of microbes on a matrix depends upon selection of a suitable matrix. Nine inexpensive matrices were evaluated for their effectiveness in terms of their cell immobilization potential. Bacterial cell mass immobilized on the matrices was estimated as total proteins and then converted to dry weight. This method was employed as other conventional methods for estimation of cell density were not suitable. Results on potentials of various matrices in terms of immobilized cell densities are presented in Table 4. Activated alumina (AA) retained maximum immobilized cell density compared to other matrices and was selected for further studies.

Table 4 Density (mg/g of matrix) of immobilized *P. aeruginosa* on solid matrices

Matrix	*P. aeruginosa* cell density (mg/g)
Activated alumina	92.20
Silica gel	48.30
Coal	32.55
Sand	31.98
Coconut shell	24.60
Rice husk	24.40
Glass pieces	21.00
Refractory bricks	18.30
Rashig rings	16.56

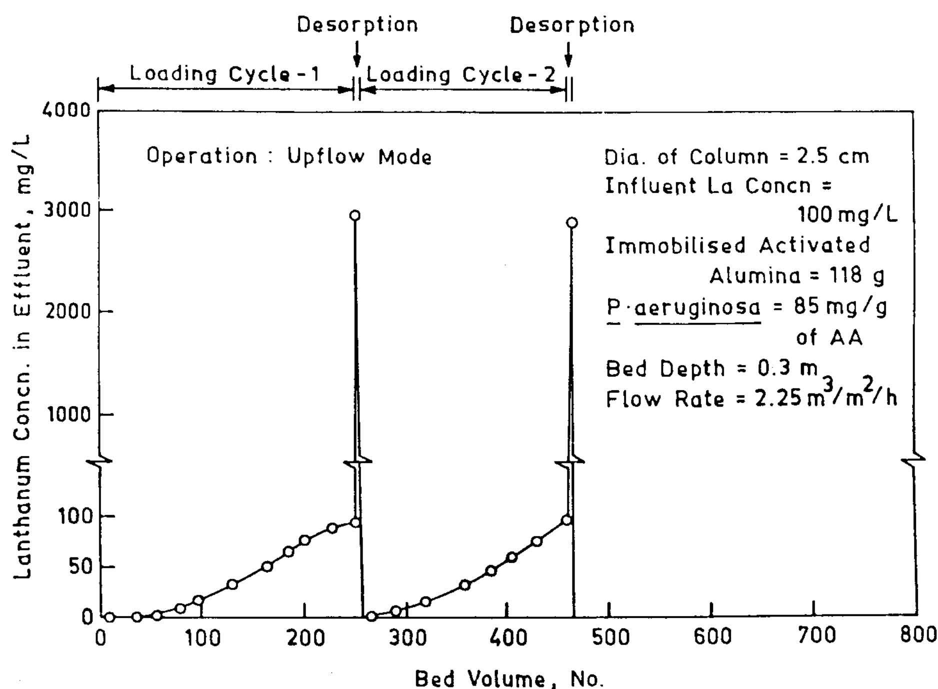

Figure 6 Recovery of La from immobilized *P. aeruginosa* and its reuse for La sorption (up-flow mode).

Column study with lanthanum

A column of 25 mm diameter and 500 mm long was packed with *P. aeruginosa* immobilized on 118 g of activated alumina. The bacterial density was 74 mg/g. Medium depth was kept at 30 cm. The column was challenged with 100 mg/l lanthanum solution with the pH adjusted to 5.0 using 0.05 M acetate buffer. The column was operated in the upflow mode at a flow rate of 2.25 $m^3/m^2/h$ (18 ml/min.).

Figure 6 presents the breakthrough curves of an immobilized microbial reactor in the first and second cycles. The immobilized column produced around 250 bed volumes of treated effluent till it reached breakthrough (C_e=0.95 C_o), whereas the column containing only activated alumina produced only 140 bed volumes. Exhausted columns were regenerated using 0.2 M citrate buffer (pH 4.0, 5 bed volumes) and then reused for lanthanum removal. About 85% of the metal adsorbed was desorbed in 1/50th of its throughput volume. The efficiency of the bioreactor in the second cycle was 80% of the first cycle.

To find out the stability of biomass on the support medium after the first cycle of column operation, total protein content was estimated using a representative sample of the adsorbent. The adsorbent could retain 95% of its original protein content, i.e., 70 mg of biomass/g. But the INT-dehydrogenase activity was lost after the first cycle. This may be one of the reasons for the reduced capacity of the column in the second cycle.

Conclusions

Adsorption of uranium (U) and lanthanides (La, Pr, Nd, Eu and Dy) by *P. aeruginosa* showed that sorption kinetics were biphasic. Two surface groups on the cell wall might be responsible for such an event. The maximum metal uptake capacity of *P. aeruginosa* was around 1 mM/g for almost all lanthanides. Around 85% of adsorbed metal was released using citrate buffer (pH 4, 0.2 M), whereas HCl could desorb 95% of the sorbed metal. EDAX studies revealed that adsorption of U and La were accompanied by partial release of Ca and Mg ions from the cell wall. A bioreactor using immobilized *P. aeruginosa* could be used to remove La from the effluents effectively.

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